

The 34th
Plant
Development
Workshop

List of Contributors
&
Program

November 4, 2000
Department of Botany
University of Toronto

34th Plant Development Workshop

November 4, 2000
University of Toronto, Toronto, Ontario.

8:45 Registration and coffee, Earth Sciences Center lobby.

9:15 Oral Presentations, Koffler Center, Rm. 108.
Welcome – Nancy Dengler
Sessional Chair – Gordon Lemon

9:20 Gordon D. Lemon^{1*} and Usher Posluszny². ¹Department of Botany, University of Toronto, Toronto, ON, M5S 3B2. ²Department of Botany, University of Guelph, Guelph, ON, N1G 2W1.
Evolution and development of the duckweed (Lemnaceae) frond.

9:30 Connie L. Soros* and Usher Posluszny. Department of Botany, University of Guelph, Guelph, ON, N1G 2W1.
Relationships in the Hydrocharitaceae – a molecular and morphological perspective.

9:45 Usher Posluszny* and Jack B. Fisher. Department of Botany, University of Guelph, Guelph, ON, N1G 2W1 and Fairchild Tropical Garden, 11935 Old Cutler Rd, Miami FL, 33156, USA.
Solving the mystery of the hook of climbing ylang-ylang (*Artabotrys hexapetalus*): a developmental study.

10:00 L. YACOB*, and Canne-Hilliker, J. Department of Botany, University of Guelph, Guelph, ON, N1G 1M9. **Comparative floral development in two species of *Eremophila*: implications for origin of bilateral symmetry.**

10:15 Ewa Cholewa¹ and Carol A. Peterson^{2*}. ¹Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, Umea, SE – 901 83, ²Department of Biology University of Waterloo, Waterloo, ON, N2L 3G1.
Onion root development in relation to calcium uptake.

10:30 Fengshan Ma^{1,2*}, Kevin J. Stevens², Carol A. Peterson¹ and R. Larry Peterson².
¹Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1; ²Department of Botany, University of Guelph, Guelph, ON, N1G 2W1.
Phellem development in the root and stem of purple loosestrife (*Lythrum salicaria* L.)

10:45 Coffee Break, Koffler Center lobby.

11:00 *Sessional Chair – Scott Douglas*

Scott Douglas^{1,*}, George Chuck², Andrew Taylor³, Lakshmi Pelecanda¹, Ron Dengler¹, and C. Daniel Riggs^{1,1} Botany Department, University of Toronto, 1265 Military Trail, West Hill, ON, M1C 1A4. ²Department of Biology, University of California, San Diego, La Jolla, CA 92093. ³Department of Biology, Wayne State University, Detroit, MI.

The KNAT-1 homeobox gene regulates *Arabidopsis* internode and pedicel development.

11:15 C. Daniel Riggs^{1,*}, Scott J. Douglas¹, George Chuck², and Patricia Springer³. 1. Botany Department, University of Toronto; 2. Biology Department, University of California, San Diego; 3. University of California, Riverside.

Altered expression of KNAT1 in mutant genetic backgrounds and phenotypic defects in transgenic plants expressing a KNAT1 dominant negative mutant.

11:30 Nela Mihajlovic and Vojislava Grbic*. Department of Plant Sciences, University of Western Ontario. London, ON, N6A 5B7.

Transition to reproductive development in *Arabidopsis thaliana*.

11:45 Denny G. Mellersh* and Michele C. Heath, University of Toronto, Toronto, ON. **A requirement for plasma membrane-cell wall adhesion in the expression of defense responses to fungal penetration.**

12:00 Lori Ann Korol* and John S. Greenwood. Department of Botany, University of Guelph, Guelph, ON, N1G 2W1. **Designing a construct for antisense repression of a programmed cell death-related cysteine proteinase gene in *Vicia faba*.**

12:15 Arunika Gunawardena^{1,3,*}, Deborah Pearce¹, Mike Jackson² and David Evans¹, 1. School of Biological and Molecular Sciences, Oxford Brookes University, Oxford, UK. 2. IACR-Long Ashton Research Station, University of Bristol, UK. 3. Faculty of Agriculture, University of Peradeniya, Sri Lanka.
Programmed cell death and aerenchyma formation in maize roots.

12:30 Lunch and Poster Presentations, Earth Sciences Center lobby.

1:30 Oral Presentations continue - Koffler Center, Rm. 108.
Sessional Chair – Julie Kang

Julie Kang* and Nancy Dengler. Department of Botany, University of Toronto, Toronto, ON, M5S 3B2.

The pattern of cell cycling in developing provascular strands of *Arabidopsis* leaves.

1:45 Jim Mattsson* and Thomas Berleth. Department of Botany, University of Toronto, Toronto, ON, M5S 3B2.

The role of auxin in leaf vascular pattern formation

2:00 Danielle Vidaurre*, Sara Ploense and Thomas Berleth. Department of Botany, University of Toronto, Toronto, ON.
The *amp1* mutant acts as a suppressor of the *mp* mutant.

2:15 Najeeb U. Siddiqui*, Clare A. Hasenkampf and C. Daniel Riggs. Department of Botany, University of Toronto, Toronto, ON.
Expression of a Chromosome Condensation Factor During Mitosis and Meiosis in *Arabidopsis*: Phenotypic abnormalities in the Shoot Apical Meristem by Antisense Inhibition.

2:30 Siobhan Brady* and Peter McCourt. Department of Botany, University of Toronto, Toronto, ON. M5S 3B2.
The Role of ABI3 in *Arabidopsis* development.

2:45 Krogan, N.T.* and Ashton, N.W. University of Toronto, Toronto, ON.
The Isolation and Analysis of MADS-box clones from the bryophyte, *Physcomitrella patens*.

3:00 Coffee Break, Koffler Center lobby.

3:15 Keynote talk:
Christian S. Hardtke, Jim Mattsson, Danielle Vidaurre, George Stamatiou, Sasha Singh, Naden Krogan, Rachel Lau, Thomas Berleth*. Department of Botany, University of Toronto, Toronto, ON, MSS 3B2.
Auxin Transport and Auxin Response Transcription Factors in Plant Morphology and Vascular Development

4:15 Wine and Cheese, Earth Sciences Center lobby.

Poster Presentation Abstracts

Eisho Nishino and Nancy Dengler. Faculty of Horticulture, Chiba University and Department of Botany, University of Toronto. **Leaf structure and growth of wild type and three mutants of Japanese morning glory.**

We studied three leaf shape mutations (*maple*, *delicate*, and *maple*^{wl}/*delicate* double mutant) that have been known in Japan for about 200 years. The genetics of these mutants was studied in the 1930s, and interest has been renewed as a result of recent molecular biology findings. Up to now, however, no detailed analysis of the structure and development of these mutant phenotypes has been made. We have initially characterized leaf shape, anatomy, and growth characteristics with the long-term goal of understanding the role of these genes in leaf development. The mutant phenotypes differed from wild type in lobe number, lobe length, sinus depth, and vein angle. *maple* and *delicate* were similar to wild type in cross sectional anatomy and in epidermal cell size and shape. In the double mutant, the lamina is highly reduced, and epidermal cells are narrower. In some double mutants, only the midrib region is present, and inverted vascular bundles occur (suggesting some loss of dorsiventrality). We measured growth of the first ten leaves of plants grown under controlled conditions and found that growth rates differed slightly among the four genotypes, resulting in different mature leaf lengths. Further analyses of mature leaf shape and development are underway.

John N.A. Lott* and Marcia West. Department of Biology, McMaster University, Hamilton, ON, L8S 4K1. Mineral nutrient reserves in dry seeds of wild type, *pho1*, *pho2* and *man1* mutants of *Arabidopsis thaliana*.

The widely used model plant *Arabidopsis thaliana* has tiny seeds with a single aleurone layer, an embryo with two cotyledons and a root-shoot axis (Keith *et al.*, 1994, *The Plant Cell* 6: 589-600). The embryo has many storage lipid vesicles and protein bodies containing mineral nutrient storage spheres called globoids (Mansfield and Briarty, 1992, *Can. J. Bot.* 70: 151-164). Globoids are spherical inclusions rich in phytate, a cation salt of myo-inositol hexaphosphoric acid and thus may serve as a store of inositol, P, K, Mg, Ca, Fe, Zn and Mn. We used energy dispersive x-ray (EDX) analysis to determine the elements present and their relative amounts in globoids of dry wild type seeds, as well as seeds of a reduced total P uptake mutant called *pho1* (Poirier *et al.*, 1991, *Plant Physiol.* 97: 1087-1093), a phosphate accumulator *pho2* (Delhaize and Randall, 1995, *Plant Physiol.* 107: 207-213) and a metal accumulator *man1* (Delhaize, 1996, *Plant Physiol.* 111: 849-855). Key findings of this study were: 1) globoids in protein bodies from nine different tissues in dry *Arabidopsis thaliana* seeds contained P, K, Mg and Ca and sometimes traces of Fe and Zn; 2) globoids contained higher Ca and lower Mg amounts than occurs in globoids in seeds of most other plant species; 3) globoids in comparable tissue/organ regions of seeds were very similar in elemental composition for wild type and all mutant plants.

Nancy F. Silva¹ §, Sophia L. Stone¹ §, Loraine N. Christie¹ §, Waheeda Sulaman^{1,*}, Katherine A. P. Nazarian², Laurie A. Burnett², MaryAnne Arnoldo², Steven J. Rothstein³, Daphne R. Goring¹. Biology Department, York University, Toronto, ON, M3J 1P3,
²Pioneer Hi-Bred Production Ltd., Canola Research Station, Georgetown, ON, L7G 4S7,
³Pioneer Hi-Bred International Inc., Trait and Technology Development, Johnston, IA 50131. **Rejection of self-incompatible *Brassica napus* pollen by *Brassica napus* cv. Westar plants expressing the S receptor kinase**

Expression of an S receptor kinase (SRK_{910}) transgene in the self-compatible *Brassica napus* cv. Westar conferred the ability of the transgenic pistil to reject pollen from the self-incompatible *Brassica napus* W1 line which carries the S_{910} allele. In one of the SRK transgenic lines, 1C, virtually no seeds were produced when the transgenic pistils were pollinated with W1 pollen (Mean seeds/pod = 1.22). This response was specific to the W1 pollen since pollen from a different self-incompatible *Brassica napus* line, T2, and self-pollinations were fully compatible. Westar plants expressing an S locus glycoprotein transgene (SLG_{910}) did not show any self-incompatibility response towards W1 pollen. Transgenic Westar plants resulting from crosses between the 1C SRK transgenic line and three SLG_{910} transgenic lines were also tested for rejection of W1 pollen. The additional expression of the SLG_{910} transgene in the SRK_{910} transgenic plants did not significantly cause a further reduction in seed production (Mean seeds/pod = 1.04) or have any detectable effects on the number of pollen grains adhered to the pistil. Thus, while the SLG gene was previously reported to have an enhancing effect on the self-incompatibility response, no evidence for such a role was found in this study.

Roger F Horton, Department of Botany, University of Guelph. **Heterophylly in *Ranunculus flabellaris*: the Effect of Blue Light**

The amphibious buttercup, *Ranunculus flabellaris*, exhibits dramatic heterophylly between emergent (land-form) leaves and the highly-dissected leaves produced underwater. These patterns of development are known to be strongly influenced by a range of environmental and plant regulator treatments. Submergence of plants in a solution of abscisic acid results in the formation of leaves with land form characteristics. The present study demonstrates that plants grown underwater in blue light will also produce land form leaves - without any regulator treatments. The photobiological and regulator controls of leaf development in *R. flabellaris* and other species are discussed.

S. Singh, C. Hardtke and T. Berleth. University of Toronto, Toronto, ON. **The mp;nph4 double mutant reveals overlapping gene function in pattern formation during embryogenesis in *Arabidopsis thaliana*.**

To date, there exist only three mutants (published) in the ARF gene family in *Arabidopsis thaliana*: *monopteros*, *nph4* and *ettin1*. ARF genes contain both DNA binding and protein-protein interaction domains, and have transcriptional activity. It has been postulated that these proteins, along with the IAA family, work together to mediate gene control in response to auxin. How these proteins interact at the transcriptional level remains debatable. The protein-protein binding domains which are common to both ARF and IAA members imply that dimerization plays a role in the transcription of auxin-inducible genes. The *nph4* single mutant looks essentially normal but is impaired in auxin perception, which is revealed by hypocotyl growth curvature assays. By contrast, mutations in *MONOPTEROS* (*MP*) interfere with the development of the root, hypocotyl and vascular tissue patterning, which yields a seedling-lethal phenotype. Both mutants are linked to auxin insensitivity. The double mutant of *mp;nph4* reveals additional regulatory functions of *NPH4*, which are masked by redundant *MP* function in the *nph4* single mutant. In about 50% of the double mutant embryos, there is no formation of the cotyledons and elongated vascular strands are entirely missing. These observations assign entirely new function to *NPH4* in embryo pattern formation.

Veronica M. Koehl and Tammy L. Sage. Department of Botany, University of Toronto, 25 Willcocks St., Toronto, ON M5S 3B2. **Characterization of the Pollen Tube Pathway in *Aristolochia elegans* (Aristolochiaceae).**

The structure of the pollen tube pathway in unpollinated and pollinated carpels of *Aristolochia elegans* was characterized. Following pollination, pollen grains germinate within anther locules in a nutrient rich medium of tapetal secretions and degenerate cells. Germinated tubes grow laterally over anther tips and subsequently travel down the interior of the funnel shaped gynostemium en route to the short, wet, hollow style and long, inferior ovary and anatropous ovules. Along the entire pollen tube pathway, tubes grow in a copious extracellular matrix (ECM). Immunocytochemical studies (using monoclonal antibodies) at the TEM level indicate the ECM to be rich in arabinogalactan proteins (AGP's). AGP's were also abundant within sub-epidermal stylar and placental cells and in pollen tube walls and cytoplasm. Monoclonal antibodies to esterified and low esterified homogalacturonans (pectins) do not localize to the ECM but are localized to the cell walls of transmitting tissue and pollen tube walls. Immunocytochemical observations support histochemical observations at the light microscope level. The present study provides the first confirmation of the presence of AGP's within the ECM of a primitive flowering plant taxon. These results are significant since AGP's have been hypothesized to be important in the evolution of pollen tube guidance mechanisms within the angiosperms.

Vincenza Pontieri and Tammy L. Sage. Department of Botany, University of Toronto, 25 Willcocks St., Toronto, ON M5S 3B2. **Pollen-Stigma Interactions in *Saururus cernuus* (Saururaceae) using Anhydrous Vapour Fixation and Cryofixation/freeze-substitution Techniques.**

Saururus cernuus (Lizard's Tail), a species belonging to the primitive herbaceous angiosperm family Saururaceae, exhibits high rates of self-sterility due to a self-incompatibility (SI) mechanism operating on a "dry-type" stigmatic surface. This study investigates the structural and functional aspects of pollen-stigma interactions following cross and self pollination. An unpollinated stigmatic cell at anthesis has a prominent vacuole containing calcium oxalate crystals embedded in osmophilic-positive organic matrices, and a dense cytoplasm distinguished by abundant ER, Golgi, vesicles, mitochondria, and plastids. Following a compatible pollination, pollen grains adhere to the stigmatic cells via an adhesion zone ("foot"), hydrate within 45 minutes, and germinate within 3 hours. The SI response in stigmatic papillae is rapid, and results in changes in the endomembrane system, mitochondria, and calcium oxalate crystals. Self pollen grains remain adhered to stigmatic cells via the foot layer, but they fail to hydrate and germinate. Self-pollinated stigmas resume the ground state appearance of the endomembrane system present in an unpollinated stigma by 5-8 hours following self-pollination. To gain a better understanding of the role of cellular machinery in the SI response, we are presently characterizing the ultrastructural development of stigmatic cells using standard and high voltage transmission electron microscopy in combination with quantitative imaging on cryofix/freeze-substituted- and anhydrous, vapour-fixed tissues.

MA Lortieau, BJ Ferguson, FC Guinel. Wilfrid Laurier University. **Reduction of pea nodulation by cytokinin treatment.**

Sparkle, a freezer pea, was inoculated with *Rhizobium leguminosarum* 3 days after planting (dap) and treated with benzylaminopurine (BAP) 4 and 6 dap. Concentrations ranging from 0.5µM to 25µM were used and the nodules counted 24 dap. Concentrations higher than 10µM were inhibitory and nodulation was blocked with 25µM BAP. Six day-old plants treated with similar concentrations of BAP were found to produce ethylene. A light microscopy study on 17 day-old plants was undertaken to localize the stage(s) of nodule development most sensitive to 15µM BAP. Infection threads were able to form and to enter the outer cortex. The threads grew abnormally, mostly parallel to the root surface; they formed loops and intertwined. Cell division centers were rarely seen in the inner cortex. Absorbance measurements at 600 nm showed that BAP did not have any effect on the bacteria; cultures grew well with concentrations similar to those used on plants. We propose that cytokinin, a known activator of the enzyme ACC synthase, indirectly inhibits nodulation by making the plant produce more ethylene. We tested the hypothesis by checking if BAP-treated plants form nodules following the addition of ethylene inhibitors.

Oral Presentation Abstracts

Gordon D. Lemon^{1*} and Usher Posluszny² ¹Department of Botany, University of Toronto, Toronto, ON, M5S 3B2. ²Department of Botany, University of Guelph, Guelph, Ontario N1G 2W1. **Evolution and development of the duckweed (Lemnaceae) frond.**

Shoot development in three duckweed (Lemnaceae) species (Spirodela polystachya, Lemna minor, Wolffia borealis) was studied and compared. Duckweed shoots are extremely reduced and no evidence of a shoot apical meristem was seen during development. Duckweed shoots generally consist of a single unit (a frond) that is interpreted as a developmental hybrid (of leaf and stem origin) and may best be described and conceptualized as a metamerous unit (an internode and associated node with its appendages). In the pocket(s) of older fronds, successive buds arise from meristematic tissue at the base of the previous bud. This bud development appears homologous to supernumerary bud development (multiple buds at a node) that occurs in Pistia stratiotes. First formed buds and pockets develop from tissue on the dorsal surface near the base of frond primordia. We suggest that the morphology of Lemnaceae plants can be understood as a result of the progressive simplification of shoots from Spirodela to Lemna to Wolffia, all of which have evolved from a Pistia-like shoot system.

Connie L. Soros* and Usher Posluszny. Department of Botany, University of Guelph, Guelph, Ontario, Canada N1G 2W1. - **Relationships in the Hydrocharitaceae – a molecular and morphological perspective.**

A phylogenetic analysis of Hydrocharitaceae was conducted using data from the internal transcribed spacers (ITS-1;2) of the nuclear ribosomal genes, the chloroplast gene rbcL and the flanking introns of the chloroplast matK gene. The aquatic, monocotyledonous Hydrocharitaceae consists of 17 genera which are highly variable in habitat, pollination mechanism and morphology. Numerous convergences and reduced vegetative and reproductive structures make it difficult to interpret morphological characters for phylogenetic analysis. Our combined molecular data set showed high congruency with results published previously using rbcL data alone, or a combination of rbcL and matK (coding region) data. The combined data consistently show a monophyletic origin for the family. Other results include the monophyly of seagrass genera (*Enhalus*, *Halophila* and *Thalassia*), a possible sister group relationship of Najadaceae and Hydrocharitaceae, and several well-supported monophyletic clades within the family. Currently, morphological and developmental characters within the Hydrocharitaceae are being investigated to supplement the molecular data for further investigation of relationships.

Usher Poslusny* and Jack B. Fisher. Department of Botany, University of Guelph, Guelph, ON, N1G 2W1 and Fairchild Tropical Garden, 11935 Old Cutler Rd, Miami FL, 33156, USA.
Solving the mystery of the hook of climbing ylang-ylang (*Artabotrys hexapetalus*): a developmental study.

Artabotrys hexapetalus (L.f.) Bhand. is widely planted in the tropics and is known as 'climbing ylang-ylang', an ornamental liana or woody climber. New natural sprouts, or water shoots, and those induced by the damage of Hurricane Andrew (August 24, 1992) were collected and fixed in FAA. The development of lateral plagiotropic and orthotropic shoots was studied using both epi-illumination light microscopy and SEM. A series of buds develops in the axils of leaves on the orthotropic shoot. At the lateral margins of the axillary shelf, plagiotropic shoots form that will either develop into vegetative shoots, thorns, or shoots that bear hooks and flowers. The nature of the branches that develop the climbing hooks has been variously interpreted as either sympodial or monopodial. It's only through a developmental study that one can clearly see that the branch is sympodial and that the incorrect claim of monopodial growth was based on a very unusual quirk of differential growth in hook formation.

L. YACOB, and Canne-Hilliker, J. Comparative floral development in two species of *Eremophila*: implications for origin of bilateral symmetry. Department of Botany, University of Guelph, Guelph, Ontario, N1G 1M9.

The largest genus of Myoporaceae, *Eremophila*, occurs solely in Australia. *Eremophila glabra* is bird-pollinated. The calyx is bilateral from initiation to anthesis with the two lateral lobes reduced in size. Initiation of petals proceeds from abaxial to lateral and lastly adaxial. At anthesis the corolla is bilateral and conspicuously bilabiate, the upper lip composed of the two adaxial lobes and two lateral lobes. The lower lip is formed by the median abaxial lobe only and is strongly reflexed. The abaxial stamen pair initiates first then the adaxial pair. *Eremophila drummondii* is bee-pollinated. The calyx, is bilateral throughout development with the two lateral lobes reduced in size. Petal primordia initiate essentially simultaneously. At anthesis the upper lip is composed of two adaxial lobes; the lower lip is composed of two lateral lobes and the median lobe. This results in a bilateral corolla that is weakly bilabiate with lobes of almost equal size. The corolla tube is not oblique, as in *E. glabra*. Stamens initiate abaxially to adaxially resulting in didynamous stamens.

Ewa Cholewa¹ and Carol A. Peterson^{2*}. ¹Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, Umeå, SE 901 83, ²Department of Biology University of Waterloo, Waterloo, ON, N2L 3G1. **Onion root development in relation to calcium uptake.**

Calcium uptake into roots is often described as apoplastic, meaning that transport through the walls is involved. It is assumed that either the Casparyan bands are permeable to this ion, or that calcium entry occurs through transient apoplastic bypasses brought about by developing lateral roots, or that calcium uptake occurs at the root tip where the endodermis is immature. These ideas were tested using onion (*Allium cepa* L.) roots which did not develop laterals.

Radiolabeled calcium was supplied to three, discrete zones along the developing root, i.e. the tip where the endodermis (and xylem vessels) were immature, an older zone where the endodermis was mature, and an even older zone where the exodermis was also mature. Analysis of the plant parts showed that no calcium was transported away from the tip, and that the ion was transported to the shoots from the other two zones. Therefore, calcium was passing through layers with mature Casparyan bands. A compartmental elution study showed that the Casparyan band of the exodermis was impermeable to calcium diffusion. When inhibitors of calcium transport across membranes were applied, the amount of calcium in the stele was significantly reduced compared to the controls. Thus, all the data indicate that calcium moves symplastically, at least through those layers of cells with apoplastic barriers.

Fengshan Ma^{1,2}, Kevin J. Stevens², Carol A. Peterson¹ and R. Larry Peterson². ¹Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1; ²Department of Botany, University of Guelph, Guelph, ON, N1G 2W1. **Phellem development in the root and stem of purple loosestrife (*Lythrum salicaria* L.)**

The structure of the endodermis and phellem in purple loosestrife was studied using fluorescence and transmission electron microscopy. In the root, endodermal cells stretch tangentially with the increase in diameter due to secondary growth, which is followed by an equal division in every endodermal cell. One or two additional divisions occur in the daughter cells. Casparyan bands are sometimes developed in the newly-formed walls. An endodermis can differentiate also in stems, but the component cells do not divide and are shed after being tangentially stretched in a thickening stem. An unusual polyderm is formed in both the root and stem. The cell layer internal to the endodermis in stems and the pericycle in roots undergoes periclinal divisions to form the phellogen. A periclinal division in the phellogen cuts off a layer of phellem cells toward the outside that remain thin-walled (phelloid cells). A second periclinal division in phellogen cells cuts off another layer of phellem in which all cells form a Casparyan band; the majority of these eventually develop a suberin lamella. By repeated cell divisions of phellogen cells, several more phellem layers are formed, some layers of which form Casparyan bands and suberin lamellae. Phellogen is not produced.

Scott Douglas^{1*}, George Chuck², Andrew Taylor³, Lakshmi Pelecanda¹, Ron Dengler¹, and C. Daniel Riggs¹. 1. Botany Department, University of Toronto, 1265 Military Trail, West Hill, Ontario M1C1A4. 2. Department of Biology, University of California, San Diego, La Jolla, CA 92093. 3. Department of Biology, Wayne State University, Detroit, MI. **The KNAT-1 homeobox gene regulates *Arabidopsis* internode and pedicel development**

Members of the *knotted*-like homeobox (*knox*) gene family encode putative transcription factors hypothesized to regulate shoot apical meristem development in flowering plants. We have identified a loss-of-function mutation in the *Arabidopsis* *knox* gene KNAT-1. The mutant plant, called *christmas tree* (*xt*), is dwarfed, its pedicel lengths are dramatically reduced, and buds, flowers and siliques are oriented at a downward angle. The *xt* phenotype is similar to that observed in the mapping mutant *brevipedicellus* (*bp*), and a complementation test confirmed that the mutations are allelic. Comparison of cell and internode lengths in wildtype and *xt* plants indicates that defects in both cell division and cell elongation contribute to the mutant phenotype. Inspection of *xt* internodes demonstrates that the mutant produces a stripe of tissue that winds around the stem and terminates on the abaxial surface of pedicels. Furthermore, epidermal cells of the stripe are reduced in size and lack stomata, while subepidermal layers contain larger cells with reduced intercellular spaces and fewer chloroplasts. Increased distances between some vascular bundles in mutant stems indicate that vascular patterning is also disrupted in *xt* plants. Our results implicate a role for *knox* genes in pedicels and internodes as regulators of cell division, cell elongation and cellular patterning.

C. Daniel Riggs^{1*}, Scott J. Douglas¹, George Chuck², and Patricia Springer³. 1. Botany Department, University of Toronto; 2. Biology Department, University of California, San Diego; 3. University of California, Riverside. **Altered expression of KNAT1 in mutant genetic backgrounds and phenotypic defects in transgenic plants expressing a KNAT1 dominant negative mutant.**

KNAT1 is a homeodomain protein of *Arabidopsis thaliana* which has been postulated to be a transcription factor. We have monitored KNAT1 expression in normal plants and in mutant genetic backgrounds using both *in situ* hybridization with KNAT1 probes and GUS expression in transgenic plants carrying a KNAT1/GUS chimeric gene. These experiments demonstrated that KNAT1 is expressed in the embryo in domains destined to become the hypocotyl and rib meristem. In the mature plant, the peripheral and rib zones of the meristems, the L2 cell layer of stems, the pedicels and the carpels are sites of expression. The KNAT1/GUS construct was introduced into other regulatory mutants and misexpression of KNAT1 was observed in APETELLA2, AGAMOUS, STM, AND MONOPTEROS mutants, indicating that KNAT1 expression is influenced by these regulators. Conversely, ectopic expression of KNAT1 is associated with misexpression of PROLIFERA, which encodes the *Arabidopsis* cognate of replication licensing factor. Finally, we constructed a dominant negative mutant of KNAT1 in which the putative activation domain was deleted. Transgenic plants harboring this construct gave rise to unusual carpel phenotypes. As KNAT1 null mutants do not exhibit carpel defects, we infer that KNAT1 interacts with other proteins to play a role in carpel development.

Nela Mihajlovic and Vojislava Grbic*. Department of Plant Sciences, University of Western Ontario, London, ON, N6A 5B7. **Transition to reproductive development in *Arabidopsis thaliana*.**

Shoot apical meristem (SAM) continuously produces primordia that will give rise to lateral organs: leaves during vegetative development and flowers during reproductive development. Transition to reproductive development in *Arabidopsis thaliana* is abrupt. It involves production of flower primordia at a increased rate, elongation of internodes, and basipetal activation of axillary meristems. However, the *leafy* mutant phenotype revealed that there is a transitional stage between leaf and flower production. At this stage SAM produces primordia which divide early in development into an abaxial leaf and adaxial meristematic domain. Based on this phenotype we proposed a model for the transition to reproductive development in *A. thaliana*.

Denny G. Mellersh* and Michele C. Heath, University of Toronto, Toronto, ON. **A requirement for plasma membrane-cell wall adhesion in the expression of defense responses to fungal penetration.**

Perception of fungal penetration of the plant cell wall, and subsequent expression of wall-associated plant defenses occur before the fungus fully breaches the wall and enters the cell lumen. We have demonstrated for the first time that this process is dependent on adhesion between the plant cell wall and plasma membrane. Interference with membrane-wall adhesion reduced the frequency of callose deposition and extracellular generation of reactive oxygen species in response to biotrophic fungi, resulting in an increase in fungal penetration efficiency. Visualization of the state of membrane-wall adhesion using a plasmolysis assay revealed that rust fungi, but not powdery mildew fungi, cause a localized decrease in membrane-wall adhesion underneath the penetration point as a mechanism of suppressing penetration-related defense responses in their hosts.

Lori Ann Korol* and John S. Greenwood. Department of Botany, University of Guelph, Guelph, ON, N1G 2W1. Designing a construct for antisense repression of a programmed cell death-related cysteine proteinase gene in *Vicia faba*.

We are examining the function of a cysteine proteinase, designated *Vfcyspro*, in the development of *Vicia faba*. *Vfcyspro* is not temporally or spatially regulated, but apparently functions in all programmed cell death-related developmental events. To examine if *Vfcyspro* is essential in programmed cell death, we will transform *Vicia faba* with an antisense cDNA construct. The design of the construct to be used is reported here.

Vfcyspro cDNA will be inserted in antisense direction behind a copper-controllable promoter which, subsequently, will be cloned into a binary vector. This will allow for transformation of the *Vicia faba* cells by *Agrobacterium* and integration of this controllable construct into the genomic DNA. Using a controllable promoter system will allow repression to be induced at various stages of plant development. When the transformed plants are exposed to copper ions, the antisense *Vfcyspro* mRNA will be transcribed, will bind to the naturally transcribed *Vfcyspro* mRNA, and expression of the proteinase should be prevented. If *Vfcyspro* is essential for developmental events requiring programmed cell death, repression should perturb xylem differentiation, leaf and floral senescence and reduce the plants ability to tolerate pathogen invasion. The transformation system will also allow for integration into other genera such as *Arabidopsis*.

Arunika Gunawardena^{1,3*}, Deborah Pearce¹, Mike Jackson² and David Evans¹. 1. School of Biological and Molecular Sciences, Oxford Brookes University, Oxford, UK. 2. IACR-Long Ashton Research Station, University of Bristol, UK. 3. Faculty of Agriculture, University of Peradeniya, Sri Lanka. Programmed cell death and aerenchyma formation in maize roots.

Aerenchyma are gas spaces which enhance flooding tolerance in some plant species by facilitating gas diffusion between roots and the aerial environment. Aerenchyma in maize roots forms by collapse and death of some cortical cells in a process which can be promoted by imposing oxygen shortage or ethylene treatment.

Maize roots were grown hydroponically in 3 % oxygen, 1 ppm ethylene or 21 % oxygen (control) were analysed by combination of light and electron microscopy.

In situ TdT mediated dUTP nick-end labelling (TUNEL) suggested internucleosomal cleavage of DNA during cell death to form aerenchyma. Ultrastructural analysis by electron microscopy showed chromatin condensation was preceded by cytoplasmic changes including plasma membrane invagination, the formation of vesicles and the rupture of the tonoplast. This was in contrast to mammalian apoptosis in which chromatin condensation is the first detectable event. Later, cellular condensation, condensation of chromatin and the presence of intact organelles surrounded by membrane resembling apoptotic bodies were observed. All the events were completed before cell wall degradation was apparent.

Therefore, aerenchyma formation initiated by low oxygen or ethylene appears to be a form of PCD showing many characteristics of animal apoptosis, but with differences in the order of events.

The pattern of cell cycling in developing provascular strands of *Arabidopsis* leaves.

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This study investigated the pattern of cell cycling in: 1) provascular strand cell proliferation and in 2) differentiation of xylem and phloem cells from the provascular strands. We used a cyclin::GUS construct to determine the pattern of cell proliferation in developing provascular strands and the AtHB-8::GUS construct to characterize vascular cell differentiation in xylem and phloem from the provascular strands. We observed that the duration of cell divisions in veins is correlated to vein size order such that the rate of cell proliferation is extended in larger veins (primary>secondary>tertiary). Cell divisions became restricted to the primary vein late in leaf development. To investigate vascular cell differentiation, the AtHB-8::GUS construct was used as a marker for provascular cells. We used this construct to determine when vein pattern is formed in leaf development and to determine when provascular cells differentiate into mature xylem and phloem. A detailed characterization of leaf vein pattern development was conducted and found that vascular pattern is formed early in leaf development long before the maturation of xylem and phloem from the provascular strands. AtHB-8::GUS is present in provascular cells that have not yet differentiated, while differentiated cells do not express AtHB-8::GUS. While provascular cell proliferation and cell differentiation are separable processes, our study shows that they overlap temporally during *Arabidopsis* leaf development.

The role of auxin in leaf vascular pattern formation

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The mechanism behind the formation of leaf venation patterns is largely unknown. Known, however, is that vascular differentiation can be induced by application of auxin, and that regeneration of severed vascular strands depends on an apical to basal flow of auxin. To test the role of auxin and its flow in vascular patterning we have manipulated auxin flow in developing leaf primordia and recorded its effect on auxin distribution and vascular differentiation.

A construct containing auxin responsive elements fused to a marker gene in transgenic *Arabidopsis* plants was used as to visualize perceived auxin. Expression of the reporter gene was recorded by histochemical staining followed by DIC microscopy to visualize staining cells and elongated, interconnected provascular cells. Finally, inhibition of auxin flow was accomplished by germinating seeds on medium supplemented with auxin efflux inhibitors.

The results can be summarized as follows: The pattern of perceived auxin in developing leaf primordia is regulated in a strict spatio-temporal manner. This pattern precedes and correlates with the pattern of provascular differentiation. Likewise, auxin efflux inhibitors alter the distribution of perceived auxin, and provascular cells in a correlated manner.

In conclusion, by altering the flow of auxin, we can alter the distribution of auxin, and provascular cells in a concerted manner, suggesting that auxin flow is a central mechanism in leaf venation patterning.

Danielle Vidaurre*, Sara Ploense and Thomas Berleth. University of Toronto, Toronto, ON. **The *amp1* mutant acts as a suppressor of the *mp* mutant.**

The *monopteros (mp)* mutant of *Arabidopsis* is a seedling lethal mutant that interferes with the formation of vascular strands and with the initiation of the body axis. The *mp* mutant shows incompletely differentiated vascular strands and fails to develop a hypocotyl and primary root. Instead the *mp* mutant is only able to form cotyledons and shoot meristem. Auxin sensitivity is reduced in the *mp* mutant, although the mechanism by which MP acts in the auxin signaling pathway is not fully understood. Another mutant we are currently interested in is the *amp1* mutant. The *amp1* mutant exhibits pleiotropic effects such as a faster rate of leaf initiation and has six times the level of cytokinin than wild type. During development, the shoot meristem of the *amp1* mutant develops to an unusually large size. Given these two very different mutants, it is surprising to discover that the *amp1;mp* double mutant is able to produce a fertile plant. Somehow mutations in *AMP1* can normalize several aspects of the *mp* phenotype. *amp1;mp* double mutants form hypocotyls and roots, are fertile and have a fairly complete vascular system. The molecular mechanism by which mutations in *AMP1* can suppress those in *MP* is not clear. Two possibilities we are further investigating are: 1) both genes can interact in the auxin signaling pathway; and/or 2) excessive cell proliferation in the *amp1* mutant can compensate for some of the defects of the *mp* mutant. To distinguish between these possibilities, molecular probes are available. Among them are MP dependent auxin-inducible genes and cyclin D genes, the latter of which are induced by cytokinin. The interaction between MP and AMP1 is only one aspect of the broader goal of exploring the many different genetic and molecular interactors of MP.

Najeeb U. Siddiqui*, Clare A. Hasenkampf and C. Daniel Riggs. Department of Botany, University of Toronto. **Expression of a Chromosome Condensation Factor During Mitosis and Meiosis in *Arabidopsis*: Phenotypic abnormalities in the Shoot Apical Meristem by Antisense Inhibition**

Chromosome dynamics requires numerous enzymes and structural proteins to coordinate condensation, cohesion, recombination and resolution of entangled fibers for proper disjunction of chromosomes. The SMC (Structural Maintenance of Chromosomes) proteins play vital roles in many of these processes. We have cloned the first plant cognate of the SMC2 sub-family (AtSMC2-1) from *Arabidopsis*, whose members are involved in coordinating mitotic chromosome condensation. The AtSMC2-1 cDNA effectively rescued the chromosome condensation defective *smc2-Δ6* mutant indicating that AtSMC2-1 possibly plays a role in chromosome condensation in *Arabidopsis*. Transgenic plants harboring the AtSMC2-1: GUS transgene exhibited GUS staining in spatial and temporal patterns reflecting the mitotic activity of each tissue. *In-situ* hybridization demonstrated that AtSMC2-1 mRNA is present in meiotic cells, implying a role for these proteins in meiotic chromosome condensation. Lastly, we examined the consequences of reduced AtSMC2-1 expression to better understand the relationship between plant morphogenesis and cell division. We constructed transgenic plants carrying an antisense fragment of AtSMC2-1 gene under the control of CaMV (35S) promoter. We report some unusual phenotypes associated with the development of the shoot apical meristem.

Siobhan Brady* and Peter McCourt. Department of Botany, University of Toronto, Toronto, ON. M5S 3B2. The Role of ABI3 in Arabidopsis Development

The *Arabidopsis abi3* mutant is unable to complete late embryogenesis and severe alleles are viviparous. Through molecular characterization, ABI3 was identified as a homolog of the maize VP1 gene. VP1 is a transcription factor that contains a B3 DNA binding domain which is unique to plants. Expression studies also identified ABI3 as a seed specific transcript. However, using ABI3:GUS transgenic plants we have observed that ABI3 is expressed in vegetative tissues in meristematic regions and primarily in lateral root primordia. Using DIC optics, we have found that ABI3 expression appears to correlate with primordia initiation and ceases at lateral root emergence. Although ABI3 is insensitive to abscisic acid at the level of germination, it has never been shown to respond to ABA (Parcy et al. 1994). ABI3 expression in the lateral root is highly upregulated in response to ABA and to auxin. ABA also appears to be involved in lateral root initiation as demonstrated by quantifying lateral root density of various ABA mutants.

The Isolation and Analysis of MADS-box clones from the bryophyte, *Physcomitrella patens*

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Two MADS-box cDNAs, and the corresponding genomic sequences, were isolated from the moss, *Physcomitrella patens*. Conceptual translation of the clones reveals that the encoded MADS-domain proteins have the typical plant domain pattern (MIKC). We conclude that the MIKC pattern evolved in MADS-box genes after the separation of the plant lineage from that of fungi and animals and that it must have been present in the common ancestor to mosses, ferns, and seed plants. Phylogenetic analysis of a large subset of the sequenced plant MADS-box genes, incorporating those from *P. patens*, indicates that the bryophyte genes are not orthologues of spermatophyte genes belonging to any of the well-characterized higher plant subfamilies. Therefore, we tentatively propose that gene duplication and diversification events that created the MADS-box gene subfamilies, discernible in angiosperm and other spermatophyte groups, occurred after separation of the moss and fern lineages from that which produced higher plants. Furthermore, gene knock-out analyses suggest that the isolated moss MADS-box genes may play a role in sporophytic development and/or operate in a redundant fashion.

Christian S. Hardtke, Jim Mattsson, Danielle Vidaurre, George Stamatou, Sasha Singh, Naden Krogan, Rachel Lau, Thomas Berleth* University of Toronto, 25 Willcocks Street, Canada M5S 3B2, email: thomas.berleth@utoronto.ca. **Auxin Transport and Auxin Response Transcription Factors in Plant Morphology and Vascular Development**

The plant hormone auxin has long been known to regulate numerous responses in plants. Research in the past three years has generated supporting evidence for proposed auxin functions (1) in regular plant development. More specifically, auxin (and its polar transport) has been implicated in axis formation along the plant body (2), vascular development (1, 3, 4) and in local cell patterning events in embryos (5, 6) and meristems (7, 8). The purpose of this talk is to present some of this accumulated evidence with particular emphasis on experimental and genetic approaches in *Arabidopsis thaliana*.

Plant vascular tissues form systems of interconnected cell files throughout the plant body. Vascular tissues usually differentiate at predictable positions, but the wide range of functional patterns generated in response to abnormal growth conditions or wounding reveals partially self-organizing patterning mechanisms. Apical-basal auxin flow is presumed to align cell differentiation within the axis of the flow and to promote vascular differentiation within narrow routes of preferred auxin flow 'canals' (1). From this it can be inferred that inhibition of the apical-basal auxin transport as well as impaired auxin perception should interfere with the formation of vascular strands. Recent experimental evidence and the phenotypes of *Arabidopsis* mutants are consistent with this interpretation. Interference with auxin transport was found to result in increased vascular differentiation and disrupted vascular strands (3), and a number of mutants impaired in auxin perception display reduced vascular systems (rev. in 4). The distribution of defects locates auxin sources to distal positions in the shoot. Using auxin response reporter gene expression patterns, it is possible to visualize 'canalized' auxin flow at early stages of plant organ development. Molecular genetic screens in *Arabidopsis* have further identified presumptive auxin transport proteins (9) as well as proteins required for their polar localization (10), which should enable direct molecular tests in the future.

Mutant analysis further implicates auxin flow in embryo axis formation and meristem organization. This can be illustrated in the phenotype of the *Arabidopsis monopteros* (*mp*) mutant. Mutations in *MP* not only interfere with vascular continuity at all stages of development, but also affect axis formation in the early embryo, prevent root initiation and interfere with spatial partitioning in the shoot meristem (2). *mp* mutants are auxin insensitive and the *MP* gene encodes a transcription factor binding to functional control elements of auxin inducible promoters (5, 11). This highlights the importance of

differential auxin distribution in embryo and meristem development and details of this local patterning function have been demonstrated in auxin application (7) and auxin reporter gene experiments (8). Through a highly dynamic expression pattern, *MP* can probably confer differential auxin sensitivity at these stages.

The *MP* gene belongs to a growing family of 'auxin response' transcription factors, which are believed to interact with another class of nuclear proteins (AUX/IAA proteins) in auxin dependent gene regulation (11). *MP* can act redundantly and in combination with at least one more ARF gene, *NPH4* (12).

We finally discuss potential of ARF gene expression in manipulating plant morphology and vascular patterning as well as the use of the respective mutants in dissecting auxin signaling pathways.

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